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Susceptibility of Rainbow Trout Resistant to *Myxobolus cerebralis* to Selected Salmonid Pathogens

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Abstract

Laboratory challenges of two rainbow trout strains with *Myxobolus cerebralis* triactinomyxons confirm the resistance to whirling disease of the Hofer trout strain. Although the number of fish that became infected and developed clinical disease was similar for the Hofer and the Trout Lodge strains at all challenge doses, the median spore numbers were lower at all challenge doses for the Hofer rainbow trout. Parasite challenge doses required to produce lesions of high severity were ten-fold lower for the

Trout Lodge strain (100 triactinomyxons) than the Hofer strain (1,000 triactinomyxons). Challenges of the Hofer strain with other common salmonid pathogens demonstrate that the susceptibility of the Hofer strain was similar to what would be expected for other strains of rainbow trout, either domestic or wild. These pathogen challenges provide evidence that the Hofer trout present a low risk for introducing any pathogen that might be detrimental to native or established fish populations.

Introduction

Myxobolus cerebralis is a myxosporean causing whirling disease and it was first described in Europe in 1898 among farmed rainbow trout *Oncorhynchus mykiss* (Hofer 1903). The spread of the parasite from a presumed origin in Eurasia throughout Europe and eventually to North America is suspected to be associated with the movements of live and frozen fish (Hoffman 1970, 1990). That the disease could have impacts on wild populations of trout was originally proposed by Yoder (1972) and was realized in 1990 when fisheries biologists detected significant declines in populations of wild rainbow trout in Montana and Colorado (Vincent 1996, Nehring & Walker 1996). The severe declines in these wild rainbow trout populations stimulated a renewed interest in finding solutions for whirling disease not only for cultured fish, but for wild populations as well. Following the discovery of the two host life cycle of the parasite Wolf & Markiw (1984) both laboratory and field studies have greatly increased our knowledge of both the fish and oligochaete hosts (Hedrick 1998, Hedrick et al. 1998, Bartholomew & Wilson 2002). An area of particular interest has been the search for naturally acquired resistance to the parasite among strains of rainbow trout in North America. Field and laboratory studies to

date however, have demonstrated no more than marginal resistance to the parasite among the many stocks of rainbow trout in North America (Hedrick et al. 1998, 1999a,b, 2001, Thompson et al. 1999, Densmore et al. 2001).

Recently, a population of rainbow trout from Germany (Hofer strain) was identified as showing empirical resistance to whirling disease under hatchery conditions. This population has had presumed contact with the parasite for up to 110 years perhaps sufficient time to have developed natural resistance to whirling disease. Recent controlled laboratory challenges comparing the Hofer strain to one North American strain of rainbow trout has indeed demonstrated such resistance in the rainbow trout from Germany (Hedrick et al. 2003).

Potential applications of identifying a strain of rainbow trout with increased resistance to *M. cerebralis* include identifying genetic factors responsible for the protection from the parasite and conferring these traits to North American strains of rainbow trout that might be used in restoration efforts. Specifically, restoration in areas where rainbow trout have been established outside their native range and where whirling disease has caused the decline or elimination of rainbow trout populations for sport fisheries may benefit from such resistance. However, before any applications of these fish are realized, the broader implications of introduction must be considered. Foremost, it will be necessary to provide assurance that these fish do not pose a threat to existing populations by transmitting exotic pathogens and that the development of resistance to whirling disease has not come at the expense of increased susceptibility to other microbial diseases that may be encountered in North America. The observation of

several year classes of Hofer trout in quarantine and the pathogen challenges reported here are the first steps toward addressing these concerns.

Methods

Fish

Two strains of rainbow trout were examined in these studies. Both strains were obtained as eggs from the producers in the U.S.A. (Trout Lodge) and Germany (Hofer). Both producers are certified sources of specific pathogen-free eggs. The eggs were hatched in 12°C well water either in the Fish Health Laboratory at the University of California, Davis, California, USA or the Fish Disease Laboratory at the University of Munich, Munich, Germany. Fish were fed a commercial trout ration prior to or during the experimental challenges described below.

Pathogen Challenge protocols

The relative susceptibility of the two trout strains were tested with a panel of pathogens, including viral, bacterial and myxozoan parasites. The protocols for each set of challenge trials are described individually for each pathogen tested.

Myxozoan Challenges

Myxobolus cerebralis: Rainbow trout of each strain were approximately 10 days post-hatch and 129.5 degree days in age at the time of challenge. The protocol used for the challenges and the interpretation of the results were identical to those reported by Hedrick et al. (1999a,b). Briefly, two replicate groups of 30 fish of each strain of

rainbow trout were challenged with either 0, 10, 100, 1000, and 10000 triactinomyxons of M. cerebralis in a static bath for 2 h. Following the exposure, the fish were returned to 20 L aquaria receiving 15°C well water. At 5 mo post exposure, 5 fish from each aquaria were removed, euthanized with an overdose of anesthetic, weighed and the head removed. The head was separated into two equal halves on a midsaggital plane. One half of the head was examined for concentrations of myxospores and the other half head was placed in 10% neutral buffered formalin for histological analysis of lesion severity. Five fish were examined for spore numbers and lesion severity from each replicate group. The methods for spore enumeration and lesion scoring have been described previously (Hedrick et al. 1999a,b, 2003).

Tetracapsuloides bryosalmonae: Rainbow trout of each strain were 180 days post-hatch (approximately 20g) when exposed to *T. bryosalmonae*, the cause of proliferative kidney disease (PKD). Exposures were conducted for 7 d in a hatchery endemic for the parasite. Thirty fish from each strain were placed in a live cage (150 X 70 X 50 cm), which allowed the water to flow through 3 mm mesh. Subsequently, fish were transferred to the laboratory and held in aquaria on 13 - 14 °C specific pathogen-free water. Fish were examined daily for clinical signs of PKD such as exophthalmia and darkening of the epidermis. At 7-day intervals, two fish from each strain were euthanized with an overdose of anesthetic and dissected. Portions of liver, kidney and spleen were fixed in 10% neutral buffered formalin for histological analysis. Parasite stages were enumerated within 11 mm^2 sections of kidney and spleen. A second portion of each tissue was collected for confirmation of infection using a *T. bryosalmonae*-specific polymerase

chain reaction (PCR) assay following the protocol of Kent et al. (1998). The experiment was terminated at 120 days post-exposure.

Ceratomyxa shasta: Challenge studies with this myxozoan pathogen were conducted by administering the myxozoan parasite *C. shasta* trophozoite stages by intraperitoneal injection into replicate groups of both strains of rainbow trout. Trophozoite stages of the parasite were generated by injecting Trout Lodge rainbow trout with ascities collected from Shasta strain rainbow trout which had contracted ceratomyxosis by natural exposures to waters containing the infective stages (Ibarra et al. 1992). Ascities were frozen in liquid nitrogen and then thawed prior to injection of rainbow trout. Once ascities was formed it was harvested into a common pool from a total of 3 rainbow trout and used for the inoculum in the challenge study that compared Hofer and Trout Lodge strains of rainbow trout. At the time of challenge the mean weights of the Hofer and Trout Lodge trout were 15 g and 11g, respectively. Three replicate groups of 10 fish of each strain of rainbow trout were injected with 0.1 ml of ascities. The parasite numbers in the inoculum were not enumerated. Ten fish of each group received injections with saline only. The fish were placed into individual 20 L aquaria receiving 12°C well water. Dead fish were removed and fresh mounts of ascities or intestinal discharges were evaluated for the presence of developmental or sporogonic stages of C. shasta by light microscopy.

Bacterial Challenge

Yersinia ruckeri: Three triplicate groups of 20 rainbow trout (age 10 months, 50 g) of each strain were maintained in 70-l aquarium prior to challenge with *Y. ruckeri*. Two

groups from each strain were injected intraperitoneally with a dose of 5 X 10^4 and 5 X 10^3 *Y. ruckeri,* respectively. One group from each strain, injected either with saline only or with saline collected from standard agarose, served as negative control. Clinical signs such as haemorrhages in mouth area and exophthalmia were noted and fish that died were processed for bacteriology. Culture of *Y. ruckeri* from the kidney, liver and spleen was done using HSF-medium (Furones et al. 1993). Samples of these tissues were fixed in 10% neutral buffered formalin histological examination. Tissue samples were collected for PCR assay, which was performed using the protocol of Argenton et al. (1996). The duration of the experiment was 30 d; at that time 15 fish were euthanized and processed as above. The remaining five fish were held an additional 30 d in order to determine if a different in the prevalence of disease could be detected.

Viral Challenges

Infectious hematopoietic necrosis virus (IHNV): The Hofer and Trout Lodge strains of rainbow trout were 1.3 g and 1.0 g, respectively at the time of the IHNV trial. Two replicate groups of 20 fish for each strain of rainbow trout were exposed to either 1.2 x 10^7 (High), 1.2 x 10^6 (Medium) or 1.2 x 10^5 (Low) plaque forming units (pfu)/ml of IHNV strain (CST 039-82). This virus isolate is a member of the M clade of IHNV predominantly found in rainbow trout in Idaho (Kurath et al. 2003). The fish were exposed in a total volume of 1 l of well water at 12°C for 1 h. Two equally sized replicate groups of each strain of rainbow trout were treated as the exposed groups except that they received only cell culture media without virus. After the exposures the flow of 12°C well water was resumed to 20 L aquaria containing the different groups of fish.

Individual dead fish were removed and frozen at -80°C except for 5 freshly dead fish from each exposure group in which the concentrations of virus were enumerated by plaque assay (Arkush et al. 2003). The experiment was terminated at 26 d post initial virus exposure.

Salmonid herpes-like virus type 1 (SalHV1): Two replicate groups of 20 fish of each strain of rainbow trout were exposed to 6.4 x 10^5 tissue culture infective dose 50% ($TCID_{50}$)/ml of SalHV1 in 2 l at well water at 10°C for 1 h. Two additional replicate groups of each strain of rainbow trout were exposed only to cell culture media. After the exposures the flow of 10°C well water was resumed to the 20 L aquaria containing the different groups of fish. Dead fish were examined immediately for the presence of the virus by isolation using the CHSE-214 and RTG-2 cell lines as described by Eaton et al. (1989). The experiment was terminated at 30 d post initial virus exposure when 5 fish from each replicate were examined for presence of the virus by isolation.

Results

Myxobolus cerebralis challenge

Both strains of rainbow trout became infected at each dose of exposure tested (Table 1). At the lowest challenge dose (10 triactinomyxons per fish) all 5 fish in both replicate groups of Trout Lodge rainbow trout were infected compared to only 3 and 4, respectively for the two replicate groups of Hofer rainbow trout. Clinical signs of whirling disease were absent among both strains of rainbow trout at the lowest challenge dose but were present among all fish sampled at the higher doses. The number of spores found were up to 10-fold less in the same dose exposures when Hofer were compared to

the Trout Lodge strain of rainbow trout (Table 2). In addition, the lesion scores were lower at 10 and 100 triactinomyxon per fish doses for Hofer compared to the Trout Lodge strain of rainbow trout (Table 2).

Tetracapsula bryosalmonae challenge

There was no mortality from PKD during the course of the experiment; however, clinical signs of the disease were observed in both strains of rainbow trout. External disease signs; exopthalmia, swollen abdomen and darkening of the skin were observed in 3/30, 5/30 and 8/30, respectively of the Hofer and in 3/30, 6/30 and 10/30, respectively, of the Trout Lodge. Internally, pale organs were evident in 11/30 Hofer and 6/30 Trout Lodge and swollen kidneys in 13/30 Hofer and 15/30 Trout Lodge. Visual examination of kidney and spleen during the first four weeks post-exposure revealed few parasites in the kidney (average =1.0 parasite/11mm^2; n=8) and none in the spleen (n=8) for both strains. Numbers of parasites were highest in the kidney during the third month post-exposure, with an average of 10.4 parasites/11mm^2 (n=8) observed in the Hofer and 4.1 parasites/11mm^2 (n=8) in the Trout Lodge strain. Numbers of parasites in the spleen remained low (average < 1.0 parasite/11mm^2) for both strains for the duration of the experiment and both strains showed decreased numbers of parasites in the kidney during the fourth month (average = 1.3 parasites/11mm^2 for Hofer; average = 3.2 parasites/11mm^2 for Trout Lodge. Detection of infection by PCR showed 100% of each strain was infected with the parasite during the first three months post-exposure; infection prevalence declined during the fourth month post-exposure to 95% of Hofer and 70% of Trout Lodge.

Ceratomyxa shasta challenge

With the exception of one fish in one replicate of the exposed Hofer strain all fish died injections with trophozoites of *C. shasta* (30/30 Trout Lodge and 29/30 Hofer). There were only 2 control fish that died both of the Hofer strain. No parasites were found in either dead control fish. In contrast, trophozoites or sporogonic stages or spores were detected in all parasite-injected fish that died. The one exposed Hofer rainbow trout sampled at the terminus of the study showed no evidence of *C. shasta* in the intestine.

Yersinia ruckeri challenge

Only three fish died following challenge with *Y. ruckeri*, all from the Trout Lodge strain that received the highest bacterial dose. At 14 d post-exposure the most commonly detected clinical disease sign was haemorrhage in the mouth area, which was evident in 9/15 Trout Lodge challenged at the low dose and 10/15 at the high dose; 1/15 Hofer strain at the low dose and 5/15 at the high dose showed this disease sign. No disease outbreak occurred in the 5 fish from either strain held an additional 30 d.

Salmonid herpes-like virus type 1 (SalHV1)

There were a total of only 3 fish that died following virus exposure. The virus was recovered from the one dead Trout Lodge trout and from one of two Hofer trout that died. All other exposed and control fish in the trial appeared healthy throughout the 30 d period. There was no virus recovered from any fish examined at the end of the trial.

Infectious hematopoietic necrosis virus (IHNV)

Mortality in all virus-exposed groups was high, ranging from 90 - 100% in the Hofer trout and 85 – 97.5 % in the Trout Lodge rainbow trout (Table 3). Virus was recovered from all virus-exposed dead fish examined. The concentrations of virus found in the virus-exposed Hofer rainbow trout ranged from 5.6×10^6 to 4.8×10^7 pfu per gram and was similar regardless of the challenge dose. Virus concentrations found in virus-exposed Trout Lodge rainbow trout ranged from 4.8×10^6 to 2.0×10^9 pfu per gram and differed little with challenge dose.

Discussion

The effects of whirling disease on rainbow trout in areas of the intermountain west have raised concerns about the future viability of these populations and stimulated research for solutions. In areas where rainbow trout are native and where there are naturally reproducing populations, reducing the effects of whirling disease should be approached using habitat restoration and by insuring that fish stocked for sport fisheries are pathogen free. However, in areas where rainbow trout fisheries have been established outside of their native range and where whirling disease has caused declines in trout populations, introduction of a selectively bred strain of rainbow trout possessing traits of local strains with whirling disease resistance genes, may serve a useful role in restoration.

The identification of naturally resistant rainbow trout in Germany (Hofer strain) offers the first opportunity for management of whirling disease using disease-resistant fish (Hedrick et al. 2003). Introductions of rainbow trout, principally as eggs, from distant origins (e.g. Tasmania, South Africa) have and continue to occur in the U.S.A.

There are both benefits and risks associated with these introductions. Of the disease risks, the introduction of exotic pathogens, which can be reduced by rigorous fish health inspections and certifications, and the unknown susceptibility of the introduced trout to endemic diseases must be considered. A third and important risk is the potential introduction of genes by crosses with established populations of trout (e.g. rainbow or cutthroat trout), a risk inherent to any stocking or restoration program in areas where wild populations of trout exist and reproduce. In areas where this risk occurs, habitat restoration is a sound approach to rebuilding populations of wild trout. However, in areas where rainbow trout populations have been lost and where there are no potential impacts on native fish species, restoration efforts with disease resistant trout should be considered, particularly strains of rainbow trout resulting from crosses that convey genes for whirling disease resistance with genes from desirable North American strains of rainbow trout. The Hofer strain is clearly more resistant to whirling disease than any strain of rainbow trout tested to date. We anticipate that crosses made with the Hofer strain will result in progeny with intermediate or equivalent resistance to whirling disease as demonstrated with *C. shasta* (Bartholomew et al. 2001; Bartholomew 1998; Ibarra et al. 1992).

To date there has been no evidence of exotic pathogens in Hofer rainbow trout reared from eggs in quarantine and rigorous fish health inspections have revealed no major pathogens. Certifications of the source of the Hofer eggs by regional and European Union competent authorities are additional criteria providing risk reductions for any exotic pathogen introduction. Also, the Hofer rainbow trout, as demonstrated in the current study, do not appear to possess an increased susceptibility to microbial pathogens

found in the U.S.A. Addressing these two key disease concerns should provide fisheries managers some security regarding potential applications of the Hofer trout in restorations efforts.

Results of experimental exposures of the Hofer and Trout Lodge strains of rainbow trout to triactinomyxon stages of *M. cerebralis* in this study support the differences in susceptibility to infection and subsequent development of gross and microscopic signs of whirling disease reported previously (Hedrick et al. 2003). Fish in this study were younger (129.5 degree-days) at challenge and differences in susceptibility were not as marked as in studies using larger fish (360 degree-days). Although the number of fish that became infected and developed clinical disease was similar for both strains at all challenge doses, the median spore numbers were lower at all challenge doses for the Hofer rainbow trout. Parasite challenge doses required to produce lesions of high severity (score = 4) were ten-fold lower for the Trout Lodge strain (100 triactinomyxons) than the Hofer strain (1,000 triactinomyxons). In the earlier study (Hedrick et al. 2003), clinical disease signs, mean spore count and lesion severity were lower at all challenge doses for the Hofer strain. Combined, these studies demonstrate that the Hofer strain develops an age-related resistance reported for susceptible strains of rainbow trout in North America (Hoffman & Byrne 1974, Markiw 1991). However, this strain develops resistance at an earlier age, giving the Hofer strain the advantage of having a more limited "window of susceptibility" than other rainbow trout strains.

The reduced numbers of spores that develop in the Hofer trout compared to other susceptible strains may also have important implications for reducing overall parasite numbers in the environment (Hedrick et al. 2003). Decreasing the number of spores

available for infecting the tubificid host has the potential for effectively lowering the infectious dose that a fish might encounter. Thus, where management for wild trout is not or cannot be practiced, this research provides opportunities for providing fish for sport fishing that will not contribute to the negative impacts of the disease.

In addition to demonstrating that the Hofer strain has developed a significant level of resistance to whirling disease, it is equally important to determine the susceptibility of these fish to other pathogens that might be encountered. To determine if during the acquisition of resistance to whirling disease resistance to other pathogens might have diminished, the Hofer strain was challenged with a selection of myxozoan, bacterial and viral agents that are known to cause disease in rainbow trout in North America. Of the myxozoans, *T. bryosalmonae* is widespread in western North America and in Europe, where it is considered an important disease of cultured salmonids (Kent and Hedrick 1986). Mortality from PKD is chronic and highly variable and is often complicated by secondary infections with other pathogens (Hedrick et al. 1986). Strain differences in susceptibility to PKD have not been reported previously and data obtained from the gross pathology, clinical signs, histology and PCR analysis of fish challenged in this study suggest that susceptibility of the Hofer and Trout Lodge strains is similar.

In contrast to the widespread distribution of *T. bryosalmonae, C. shasta* has a limited distribution, occurring only in the Pacific Northwest of the U.S. and Canada. Strain differences in susceptibility are well documented for this parasite as it causes a lethal infection that acts as a strong selection pressure on populations in enzootic areas (Bartholomew 1998). The nearly complete mortality that occurred following *C. shasta* challenge in both strains suggest that the Hofer and Trout Lodge strain have similar

susceptibilities to this parasite; however, it could be argued that a high infectious dose may have masked any differences. This demonstrated susceptibility to *C. shasta* would make this strain unsuitable for introduction into waters where this parasite is present. However, because the enzootic region for *C. shasta* overlaps the native range of rainbow trout this consideration is likely of secondary importance. The inability of the Hofer strain to resist challenge by these two very different myxozoan parasites further demonstrates the specificity of the developed resistance to *M. cerebralis* in the Hofer strain.

The only bacterial challenge was conducted using *Yersinia ruckeri* a ubiquitous pathogen that generally causes low-level mortality in rainbow trout. In the challenges conducted in this study, the Trout Lodge strain showed higher mortality, gross pathology and clinical signs than did the Hofer strain. Challenges have not yet been conducted using the agents that cause furunculosis (*Aeromonas salmonicida*) or bacterial kidney disease (BKD; *Renibacterium salmoninarum*). Rainbow trout are generally considered to have low susceptibility to furunculosis (Cipriano 1983) and if the Hofer strain does not display that trait, the risk that it could amplify and spread that pathogen should be considered. On the other hand, rainbow trout are generally considered susceptible to BKD, therefore, demonstrating the susceptibility of the Hofer strain to this disease would not present any greater risk of contracting or spreading this agent than already exists.

Susceptibility of rainbow trout to IHNV is well established and in this study both strains of trout appeared equally susceptible over the three virus challenge doses. The virus concentrations obtained from moribund fish were similar to those reported by others in rainbow trout (LaPatra et al 1989, 1990) and salmon (Mulcahy et al. 1982, 1983).

Outbreaks of IHNV are sporadic outside the Pacific Northwest and thus considerations for using this strain in areas where whirling disease is problematic would be similar to those for using other strains of rainbow trout.

In contrast to the high virulence of IHNV, SalHV1 challenge resulted in low mortality in both the Hofer and Trout Lodge strains. Low mortality and difficulty in recovering virus is not unusual as the low virulence of the virus has been demonstrated in field epidemics and experimental trials either in our laboratory (Eaton et al. 1989) and the original studies conducted by Wolf and Smith (1981). Thus, it does not appear that the Hofer strain represents any greater danger of contracting and spreading either IHNV or SalHV1 than other rainbow trout strains.

For the pathogens tested, the susceptibility of the Hofer strain was similar to what would be expected for other rainbow trout, either domestic or wild. Although the selection of pathogens for challenge is by no means complete, this suggests that the development of resistance to whirling disease for these fish did not come at the cost of increased susceptibility to other fish pathogens. During the two-year period during which these challenges were conducted, repeated health examinations were conducted on these fish with no evidence of pathogens exotic to rainbow trout in North America. This, combined with the "disease-free" certification status [specific for infectious salmon anemia (ISA), viral hemorrhagic septicemia (VHS), infectious pancreatic necrosis (IPN), IHN, and enteric redmouth] of the eggs that were imported for these studies provides evidence that these fish present a low risk for introducing any pathogen that might be detrimental to native or established fish populations. However, continued pathogen

testing will be ongoing for all groups of fish and all experiments continue to be conducted in quarantine facilities.

The most cautious approach to re-introducing/re-establishing rainbow trout populations outside of their native range should be done by using progeny from selective breeding between the Hofer strain, or other whirling disease-resistant rainbow trout, and desired and known rainbow trout broodstocks. Reducing the effects of whirling disease will require a range of solutions and potential applications of the findings presented here will differ between geographic regions depending on numerous biological, economic and political factors. However, programs to rebuild or restore fisheries should not occur at the expense of re-establishing native fish assemblages, particularly those fish species that might hybridize with rainbow trout (e.g. cutthroat trout).

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Table 1. Number of infected fish and clinical signs of whirling disease among Trout Lodge and Hofer strains of rainbow trout after exposures to graded doses of the infectious stages of *Myxobolus cerebralis* .

Species	Dose	No. infected (No. with signs)* 5 fish examined	
		Replicate 1	Replicate 2
Trout Lodge	0	0 (0)	0 (0)
	10	5 (0)	5 (0)
	100	5 (5)	5 (5)
	1,000	5 (5)	5 (5)
	10,000	5 (5)	5 (5)
Hofer	0	0 (0)	0 (0)
	10	4 (0)	3 (0)
	100	5 (5)	5 (5)
	1,000	5 (5)	5 (5)
	10,000	5 (5)	5(5)

*Black tail as recorded at the 5 mo post exposure sampling.

Table 2. Median spore numbers and lesion scores among Trout Lodge and Hofer strains of rainbow trout after exposures to graded doses of the infectious stages of *Myxobolus cerebralis* .

Species	Dose	No. infected (No. with signs)* 5 fish examined			
		Replicate 1		Replicate 2	
		Spore no.	Lesion Score	Spore no.	Lesion Score
Trout Lodge	0	0	0	0	0
	10	777,250	3	123,750	2
	100	625,000	4	216,000	4
	1,000	881,000	4	411,750	4
	10,000	330,000	4	140,000	4
Hofer	0	0	0	0	0
	10	66,250	2	55,416	0
	100	191,000	3	188,000	3
	1,000	533,000	4	294,000	4
	10,000	167,000	4	115,500	4

Table 3. Cumulative mortality among Hofer and Trout Lodge strains of rainbow trout following experimental exposures to three doses of infectious hematopoietic necrosis virus (IHNV).

	No. dead fish/no. exposed (%)	
Virus Dose	Hofer	Trout Lodge
Low (1.2×10^5 pfu/ml)	37/40 (92.5)	34/40 (85)
Medium (1.2×10^6 pfu/ml)	40/40 (100)	39/40 (97.5)
High (1.2×10^7 pfu/ml)	6/40 (90)	38/40 (95)